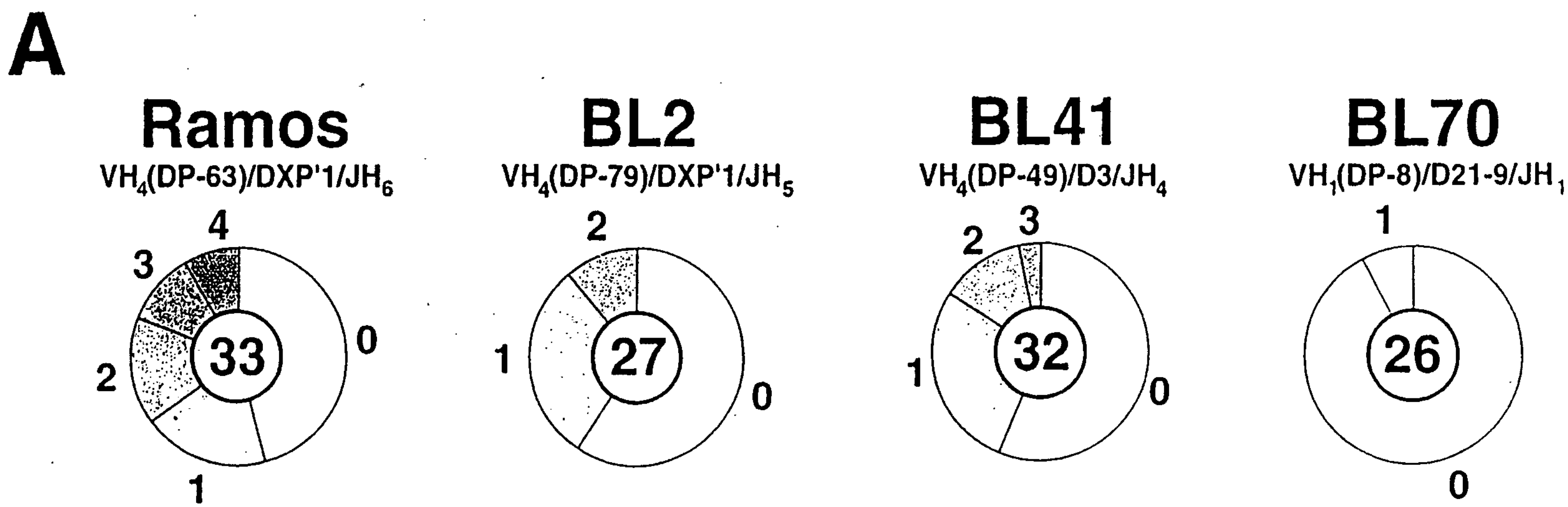

A
Ramos
VH4(DP-63)/DXP'1/JH6
4
3
2
1
0
33
BL2
VH4(DP-79)/DXP'1/JH5
2
1
0
27
BL41
VH4(DP-49)/D3/JH4
3
2
1
0
32
BL70
VH1(DP-8)/D21-9/JH1
1
0
26

FIG. 1

B

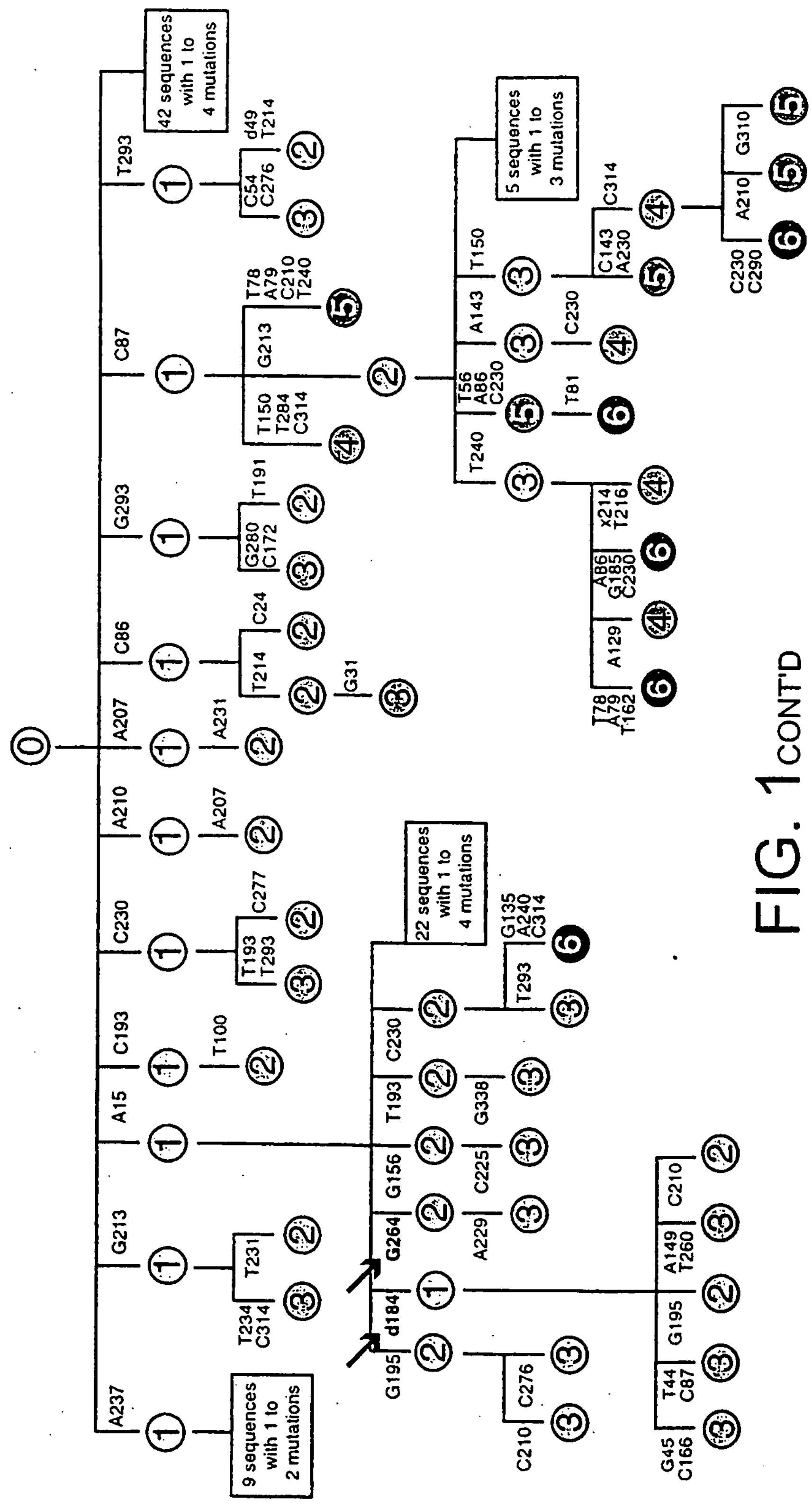
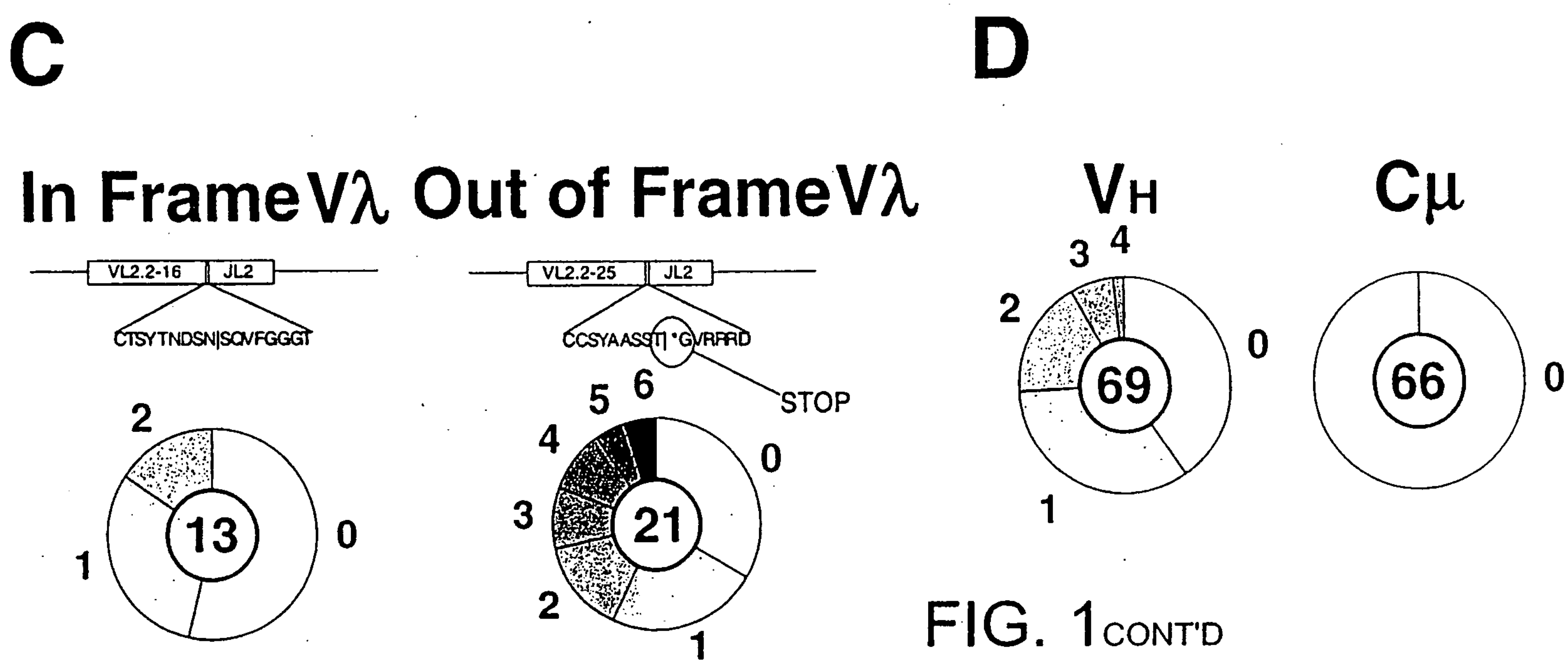

42 sequences with 1 to 4 mutations
5 sequences with 1 to 3 mutations
22 sequences with 1 to 4 mutations
9 sequences with 1 to 2 mutations

FIG. 1 CONT'D



C
In Frame Vλ
VL2.2-16
JL2
CTSYTNDSN|SQVFGGGT
13
0
1
2
Out of Frame Vλ
VL2.2-25
JL2
CCSYAASST|*GVRRRD
STOP
21
0
1
2
3
4
5
6
D
VH
69
0
1
2
3
4
Cμ
66
0

FIG. 1 CONT'D

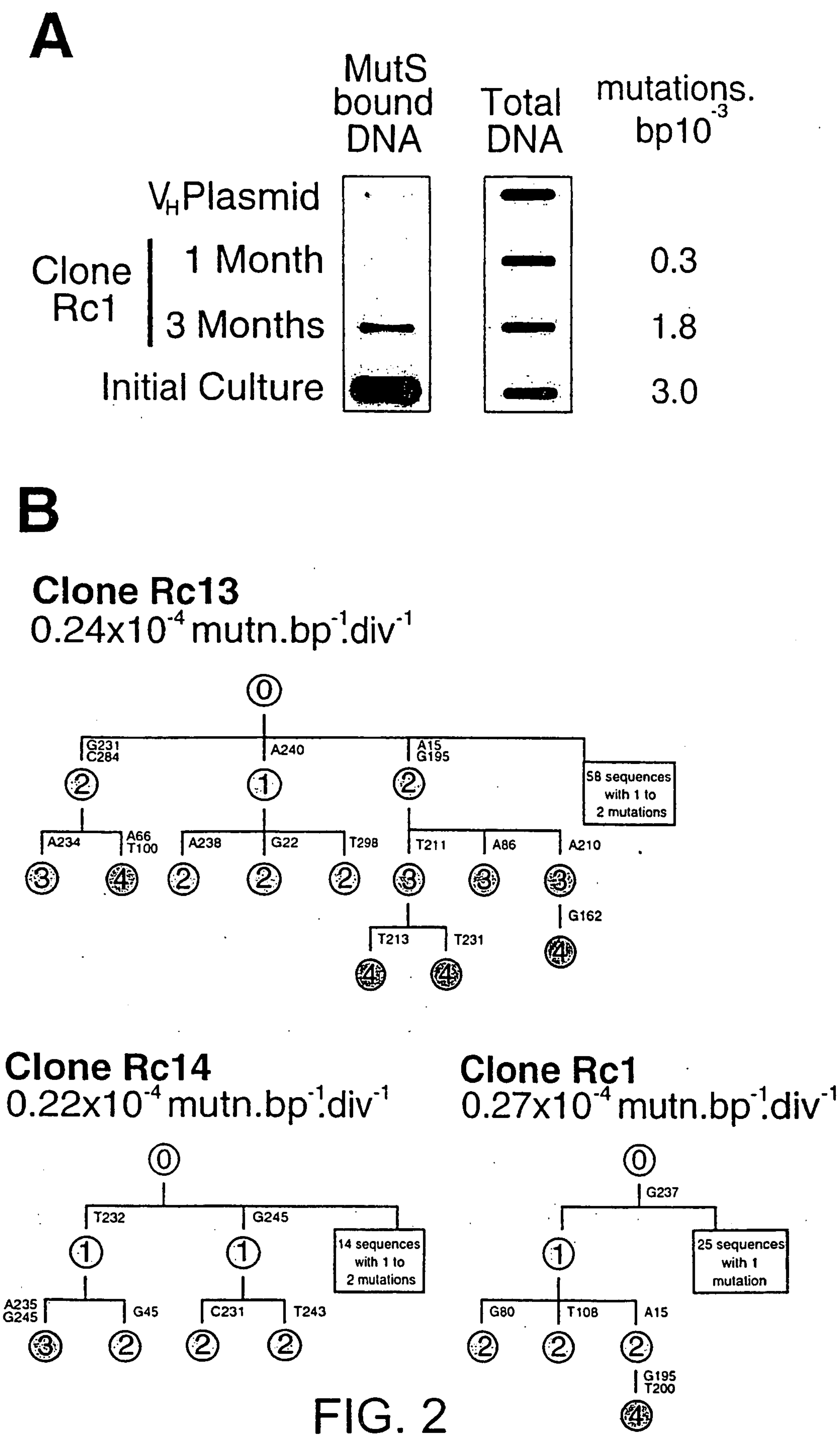

A
MutS bound DNA
Total DNA
mutations. bp10-3
VH Plasmid
Clone Rc1
1 Month
0.3
3 Months
1.8
Initial Culture
3.0
B
Clone Rc13
0.24x10-4 mutn.bp-1.div-1
G231 C284
A240
A15 G195
58 sequences with 1 to 2 mutations
A234
A66 T100
A238
G22
T298
T211
A86
A210
G162
T213
T231
Clone Rc14
0.22x10-4 mutn.bp-1.div-1
T232
G245
14 sequences with 1 to 2 mutations
A235 G245
G45
C231
T243
Clone Rc1
0.27x10-4 mutn.bp-1.div-1
G237
25 sequences with 1 mutation
G80
T108
A15
G195 T200

FIG. 2

TOTT90" ET862860

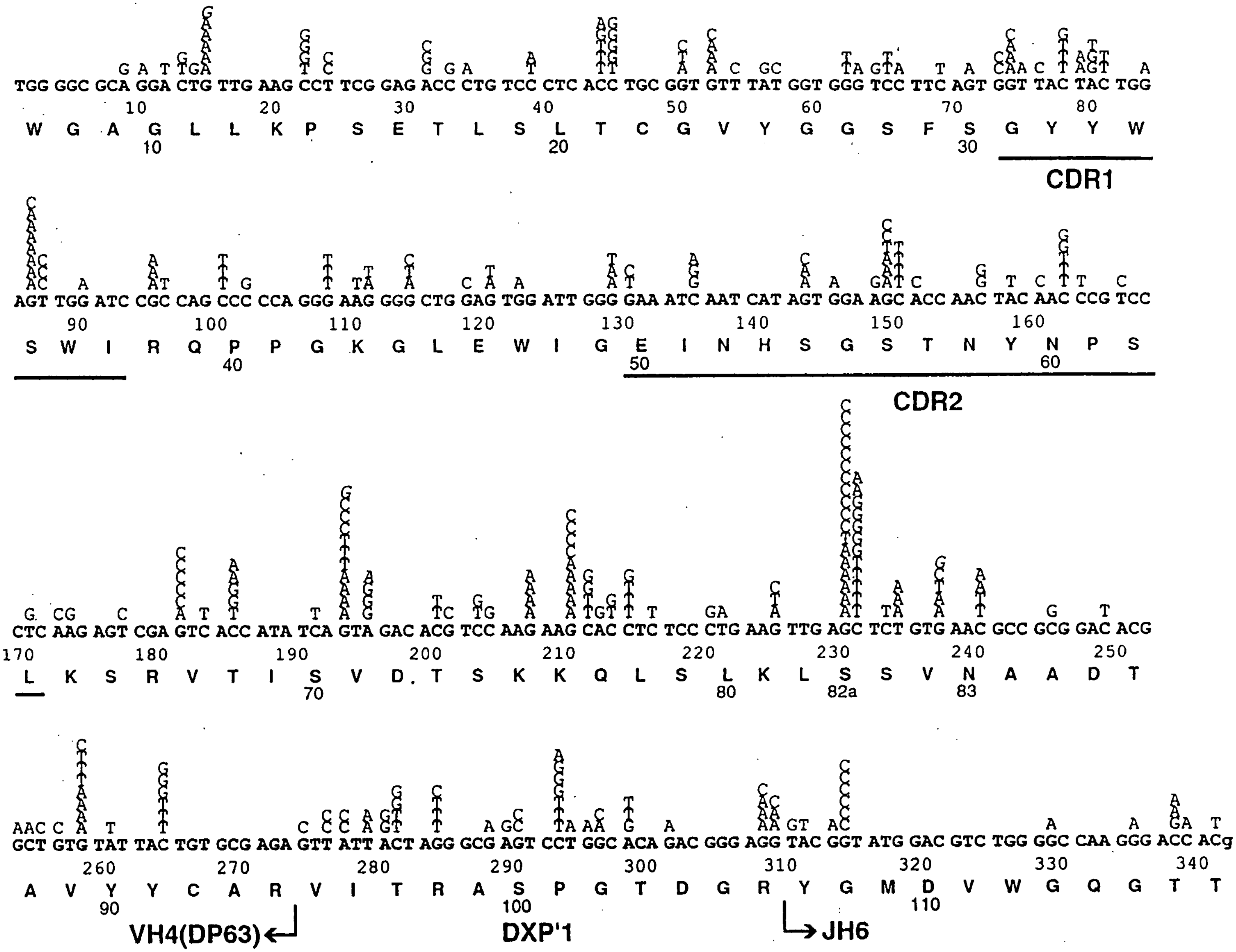


FIG. 3

A

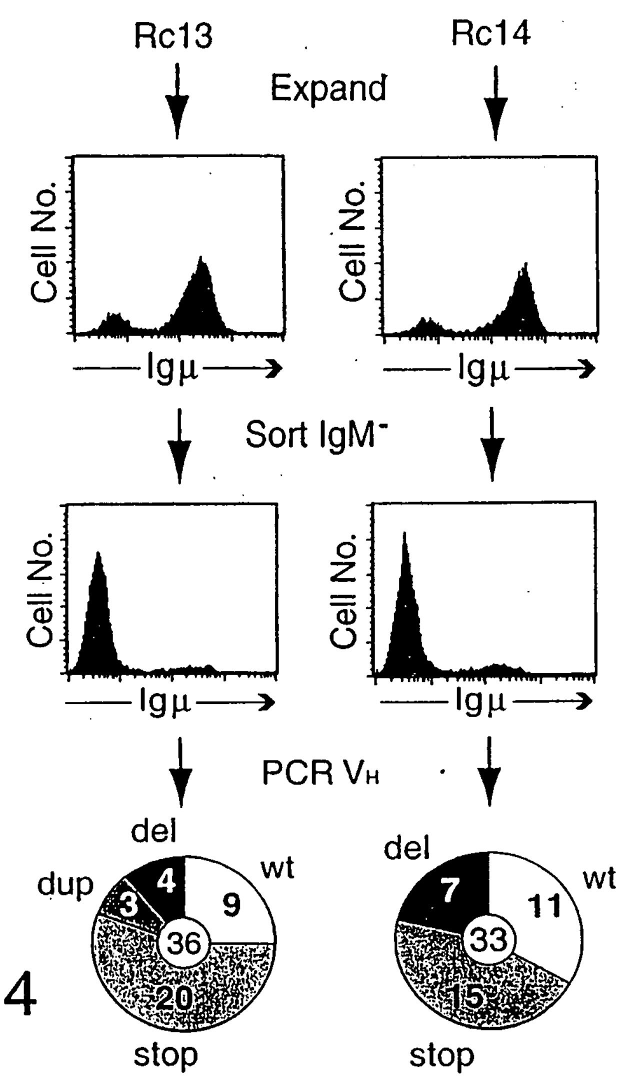


FIG. 4

B

	Rc13	Rc14
TAC 32	TAA(1;1) TAG(5;2)	TAA(1;1) TAG(1;1)
TAC 33	TAG(2;2)	
TGG 34	TGA(1;1)	TGA(1;1)
TGG 36	TGA(6;5)	TGA(1;1)
GAG 46		TAG(1;1)
TGG 47	TAG(1;1)	TGA(1;1)
GAA 50		TAA(2;2)
TCA 70	TGA(2;1)	
TAT 90		TAA(3;2)
TAC 91	TAG(3;1)	TAG(2,2)
TAC106		TAA(1;1)

C

CLONE	% REVN.	STOP	REVN.
Rc13lo.1	1.2%	TAG32	TAC
Rc13lo.2	1.1%	TAG32	TAC TGG AAG
Rc13lo.3	0.08%	TGA36	TGG
Rc13lo.4	0.01%	TAA33	TAC

D

	RGYW
TAG32	GGT**TAG**TAC
TGA36	AGT**TGA**ATC
TAA33	TAC**TAA**TGG

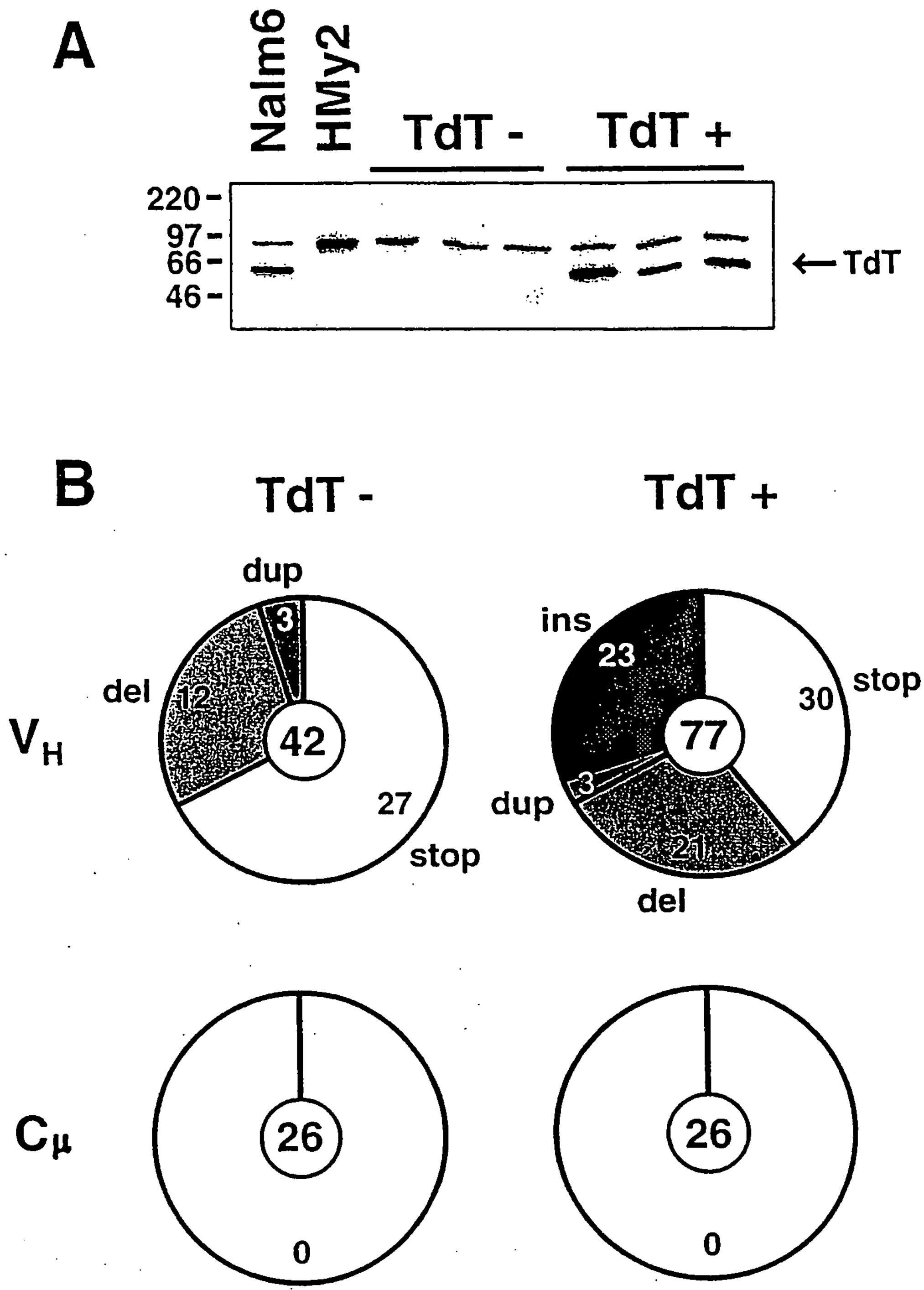

A
Nalm6
HMy2
TdT -
TdT +
220
97
66
46
← TdT
B
TdT -
TdT +
dup
3
del
12
42
27
stop
ins
23
30
stop
77
dup
3
21
del
V_H
C_μ
26
0
26
0

FIG. 5

TdT negative

Deletion

A62 GGTCCT TCAGTGG TTACTA
A120 GTGGAT T GGGGAA
A276 TATTAC TGTG.18bp.TACT AGGGCG
A306 GAGGTA C GGTATG
B93 CCGCCA G CCCCCA
B98 AGCCCC C AGGGAA
B227 TGAGCT CTGTG AACGCC
C82 TGGAGT TGGA.37bp.GAGT GGATTG
C209 AGCACC TCTCCCTGAAGTT GAGCTC
C187 ATATCA GTAGACACGTCCAAGA AGCACC
U26 CGGAGA CC CTGTCC
U199 ACGTCC AAG AAGCAC
U208 AAGCAG C TTTCTC
U268 GCGAGA GTTATTA CTAGGG

Duplication

A255 TGTGCGAGAGTTATTA CGAGAGTTATTA CTAGGG
A113 GGCTGGAGTGGATTGGG.62bp.T — ATC AGTGGATTGGG.62bp.TATC AGTAGA
U43 ACCTGCGGTGTTTAT GGTGTTTAT GGTGGG
U318 GGACGTCTGGGGCCA ACGTCTGGGGCCA AGGGAC

TdT positive

Deletion

D27 GGAGAC CCTCA CCTGCG
D31 ACCCTC A CCTGCG
D219 CCTGAA G TTGAGC
D150 CACCAA C TACAAC
D109 AAGGGG C TGGAGT
E38 CCCTCA CCTGC GGTGTT
E81 CTGGAG TTGGA..37bp..TGGAG TGGATT
E88 TGGATC CGCC AGCCCC
E93 CCGCCA G CCCCCA
E136 AATCAT AGTGGAAGCACCAACTA CAACCC
F66 CTTCAG TGGTTACTACT GGAGTT
F183 TATCAT ATCAGTA ACACGT
F215 TCTCCC TGAA.18bp.CGCC GCGGAC
F267 TGCGAG AG TTATTA

Duplication

D55 TATGGTGG.41bp.AGGG GTGG.41bp.AGGG AAGG
D123 GATTGGGGAAATCAATCATAGTGGAAGCGGAA — ATCAATCATAGTGGAAGC ACCAAC
F85 AGTTGGAT.10bp.CCCA GGAT.10bp.CCCA GGGA

Insertion (+/- Del/Dup)

D3 GGTCGC AGGACTGT / CACCC TGAAGC
D56 ATGGTGGG.50bp.CAGGG GGTGGG.50bp.CAGGG AAGGGG
D71 GTGGTT A / GGG CTACTG
D75 TTACTA C / GG TGGAGTT
D126 TGGGGA AATCAATCAT / GGG AGTGGA
D223 AAGTTG AG / GACCCGGC CTCTGTG
D232 TCTGTGAACGCCGC GCCCCCGGTCCTGTGAACGCCGC GGACAC
D235 GTGAAC GGAGG GCCGCG
D252 GGCTGT GTATTACTGT / TCC GCGAGA
D268 GCGAGA GT / AGG TATTATT
D275 TTATTA C / GG TAGGGC
D332 AAGGGA C / AG CAC
E3 GGGCGC AGGA.51bp.CTTC / GT AGTGGT
E51 TGTTTA TGGT.15bp.TACT / AGACC ACTGGAG
E80 ACTGGA G / CCC TTGGAT
E263 ACTGTG CGAGAGTTATTACT / GGTG AGGGCG
F89 GGATCC GCCAGCCCCCAGGG / AGG AAGGGG
F168 CCTCAA AGAGTCGAGT / GGG CACCAT
F195 AGACAC GTCCAAGAAG / AGGGC CACCTC
F199 ACGTCC AAGAAG / CT ACCCTGA
F242 CCGCGG ACACGGCTGTGTATTACTGT / GGA GCGAGA
F260 ATTACT GTG / CGTGA CGAGAG
F264 CTGTGC GAGAG.46bp.CGTC / ACA TGGGGC

Events with flanking single nucleotide substitutions

TdT negative

Deletion

B123 GATTGG G AAATC
C109 AAGGGT C TGGAGT

Duplication

A16 TTGAAGCCTTCGGAGT GAAGCCTTCGGAGA CCCTGT
U180 AGTCACCATATCAG ACCATATCAG TAGACA

TdT positive

Deletion

D45 CTGCGG GTTTATGGTGGGT CCTTCA
D164 CGTCCC CAAG AGTCGA
D216 CTCCCT AAG.22bp.CGGA CACGGC
E11 GACTGT T AAAGCC
E54 TTATGG GGG.25bp.GTTG GATCCG
F188 TATCAG AGACACGTCCAGAA GCACCT
F220 CTGAAG TGAGCTCTGTG AACGCC

FIG. 6

```
1/1                                     31/11
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W   G   A   G   L   L   K   P   S   E   T   L   S   L   T   C   G   V   Y   G

61/21                                   91/31
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G   S   F   S   G   Y   Y   W   S   W   I   R   Q   P   P   G   K   G   L   E
                                AGT
                                S
121/41                                  151/51
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W   I   G   E   I   N   H   S   G   S   T   N   Y   N   P   S   L   K   S   R

181/61                                  211/71
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V   T   I   S   V   D   T   S   K   K   H   L   S   L   K   L   S   S   V   N
ATC                                     CAC
M                                       H

241/81                                  271/91
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A   A   D   T   A   V   Y   Y   C   A   R   V   I   T   R   A   S   P   G   T
    TCG                                                 ACG         CAT GGC
    S                                                   T           H   G

301/101                                 331/111
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D   G   R   Y   G   M   D   V   W   G   Q   G   T   T
                            GTT
                            V
```

FIG. 7

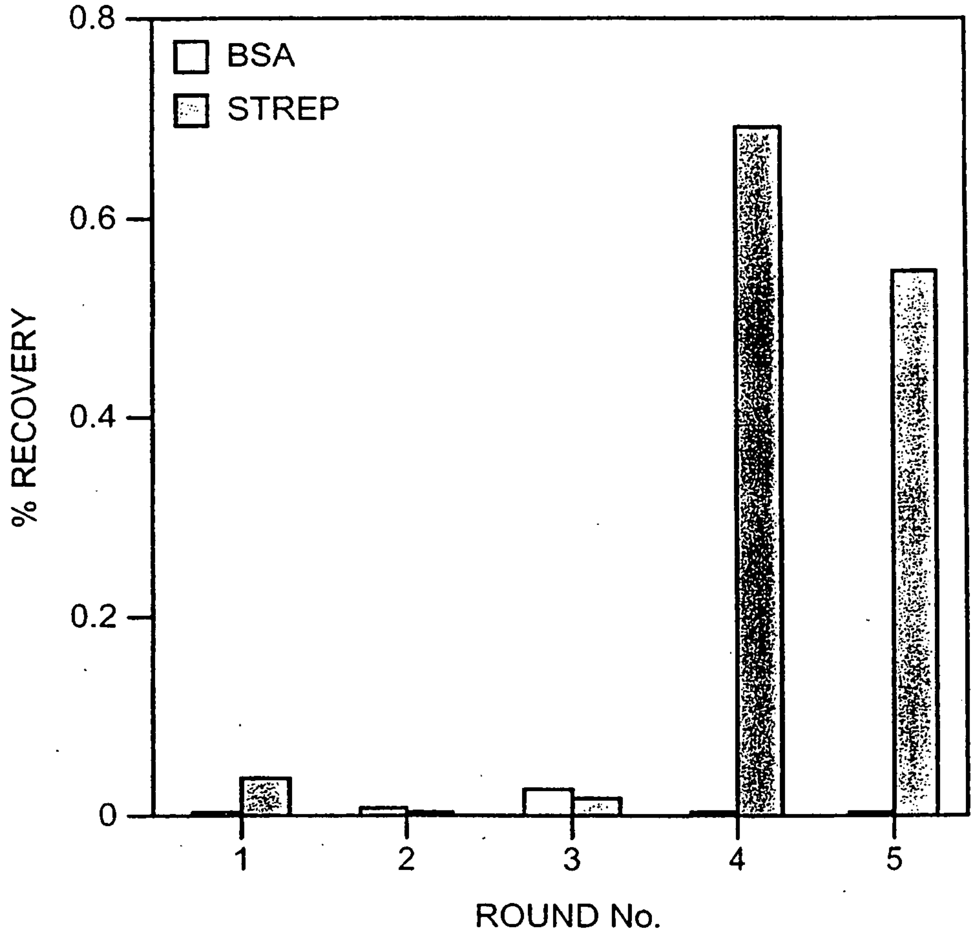
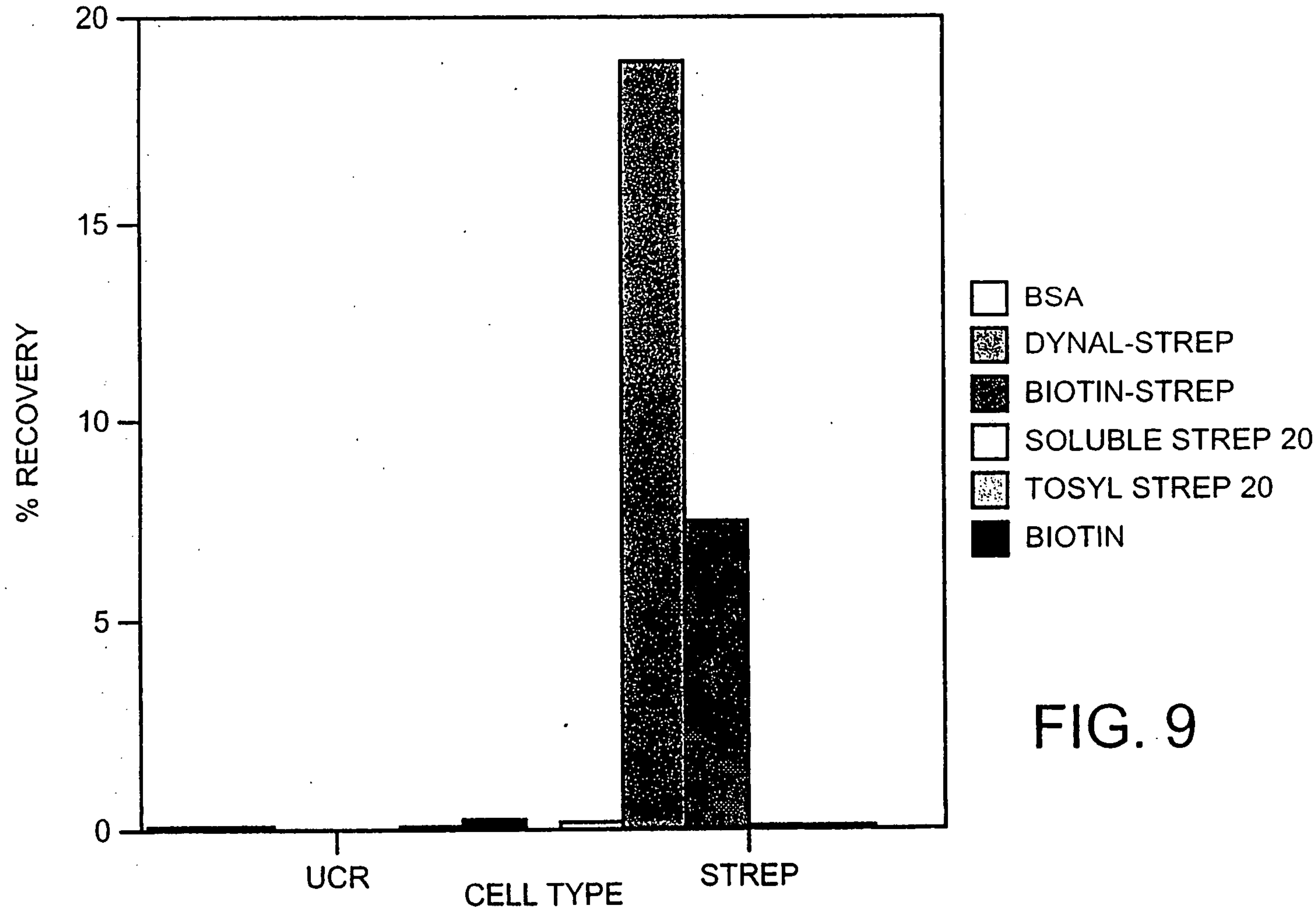
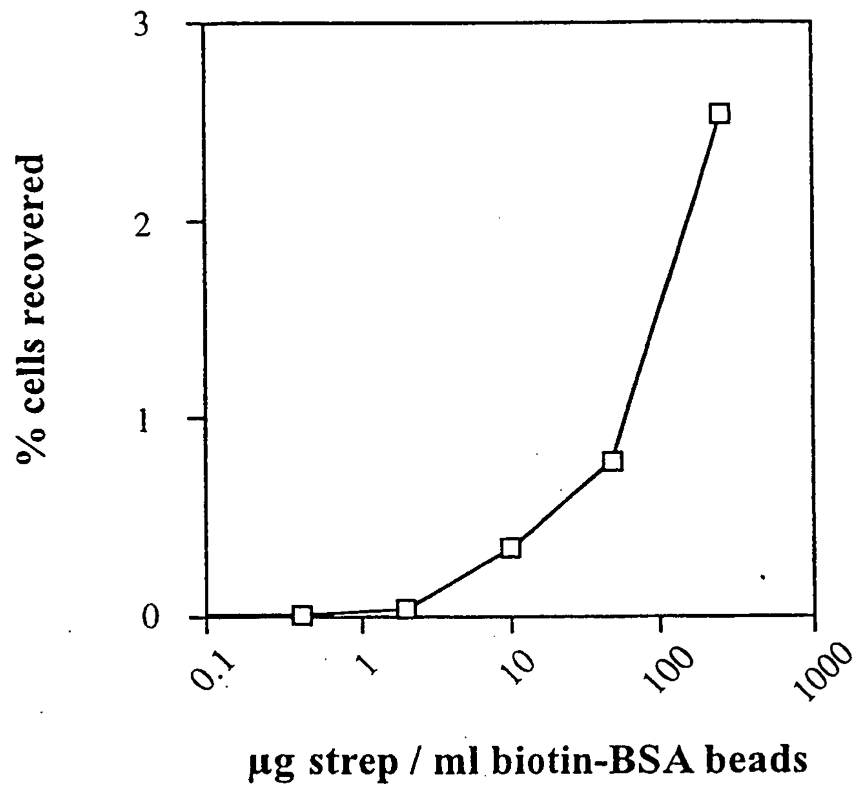


FIG. 8



20
15
10
5
0
% RECOVERY
UCR
CELL TYPE
STREP
BSA
DYNAL-STREP
BIOTIN-STREP
SOLUBLE STREP 20
TOSYL STREP 20
BIOTIN

FIG. 9



3
2
1
0
% cells recovered
0.1
1
10
100
1000
µg strep / ml biotin-BSA beads

FIG. 10

FIG. 11

VH

```
1/1                                         31/11
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W   G   A   G   L   L   K   P   S   E   T   L   S   L   T   C   G   V   Y   G

61/21                                       91/31
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G   S   F   S   G   Y   Y   W   S   W   I   R   Q   P   P   G   K   G   L   E
GGA                             AGT
G                               S
                                ATT
                                I

121/41                                      151/51
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W   I   G   E   I   N   H   S   G   S   T   N   Y   N   P   S   L   K   S   R

181/61                                      211/71
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V   T   I   S   V   D   T   S   K   K   H   L   S   L   K   L   S   S   V   N
                                        CAC                     AAC
                                        H                       N

241/81                                      271/91
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A   A   D   T   A   V   Y   Y   C   A   R   V   I   T   R   A   S   P   G   T

301/101                                     331/111
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D   G   R   Y   G   M   D   V   W   G   Q   G   T   T
        AGC
        S
```

VL

```
1/1                                                         31/11
CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC
P   A   S   V   S   G   S   P   G   Q   S   I   T   I   S   C   T   G   T   S
                TAT
                Y
61/21                                                       91/31
AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC
S   D   V   G   G   Y   N   Y   V   S   W   Y   Q   Q   N   P   G   K   A   P
        TTT TGT
        F   C
121/41                                                      151/51
AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC
K   L   M   I   Y   D   V   S   N   R   P   S   G   I   S   N   R   F   G   S
                            AAT                                 CGA TTA
                            N                                   R   L
181/61                                                      211/71
TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT
S   K   S   G   N   T   A   S   L   T   I   S   G   L   Q   A   D   D   E   A
                                    ATC
                                    I
241/81                                                      271/91
GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC
D   Y   Y   C   T   S   Y   T   N   D   S   N   S   Q   V   F   G   G   G   T
                            ACT
                            T
```

FIG. 11 CONT'D

FIG. 12

IgM in supernatant

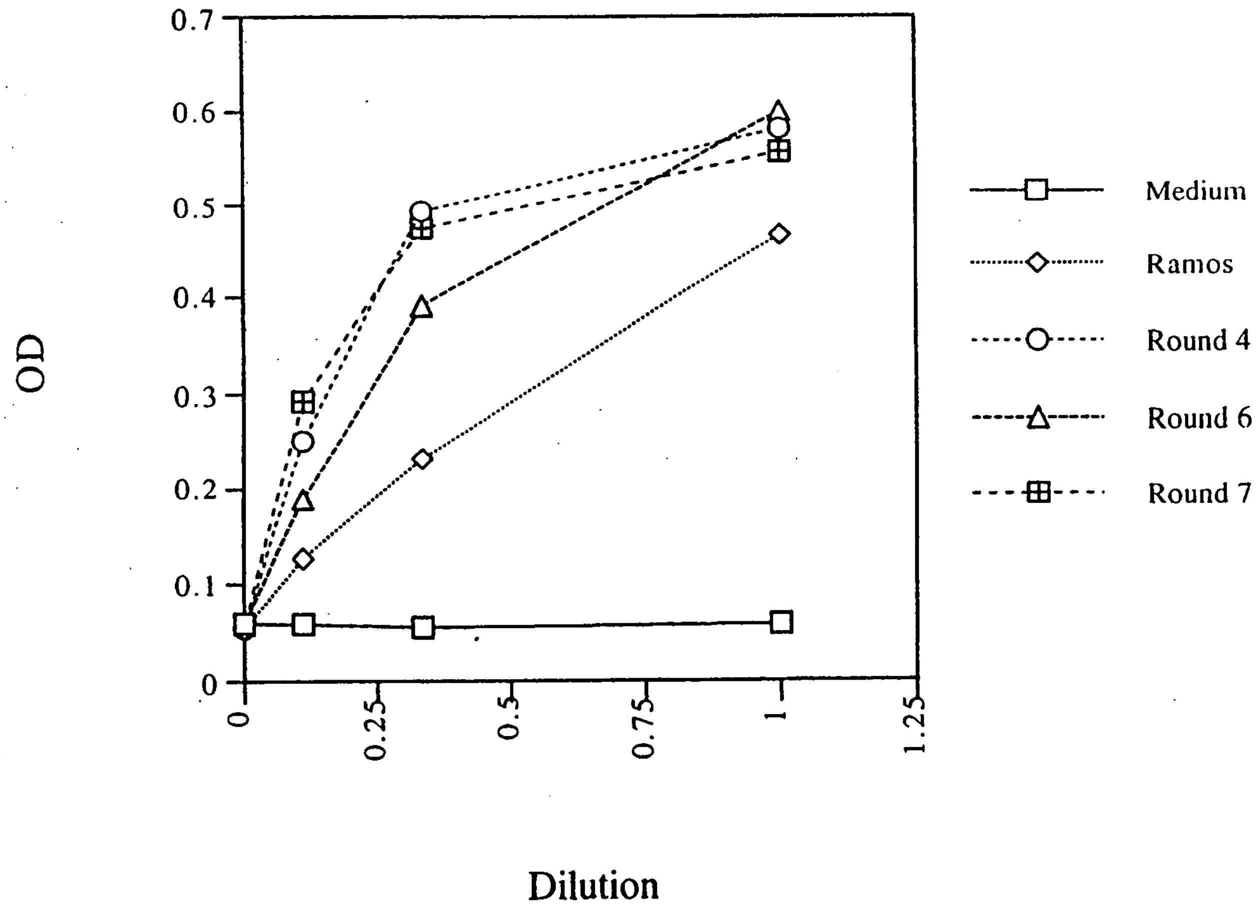


FIG. 13

Streptavidin binding of Supernatants: ELISA

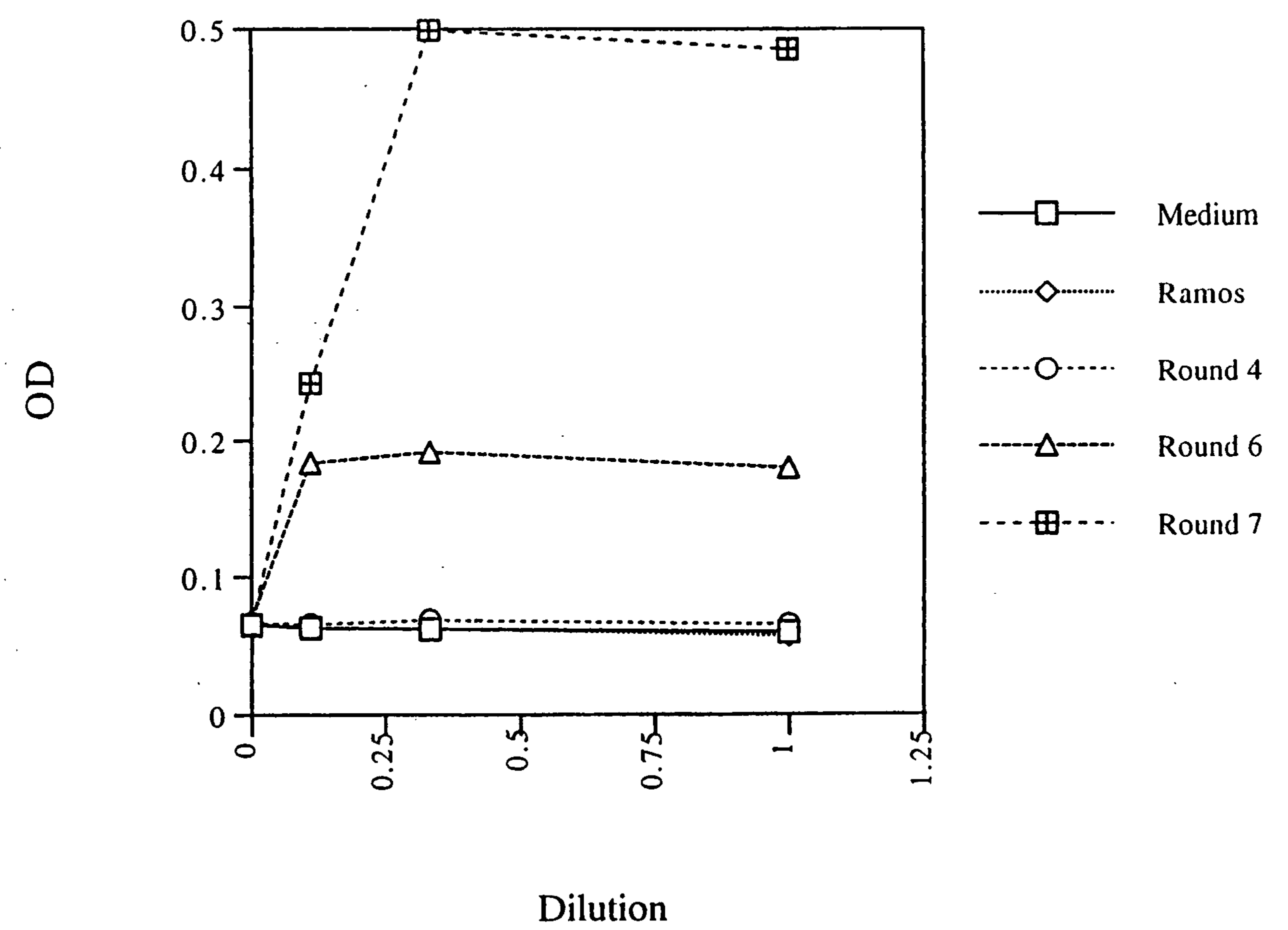
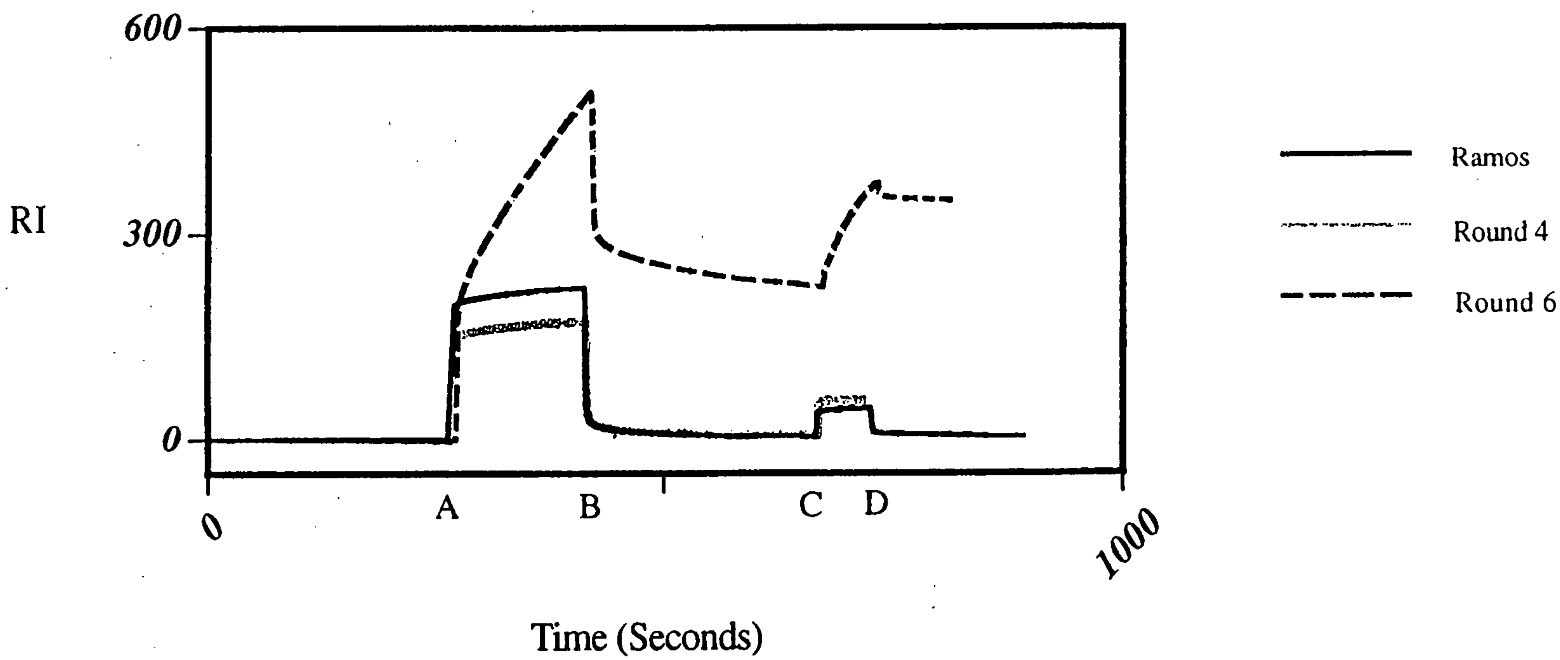


FIG. 14

Streptavidin binding of Supernatants: Biacore



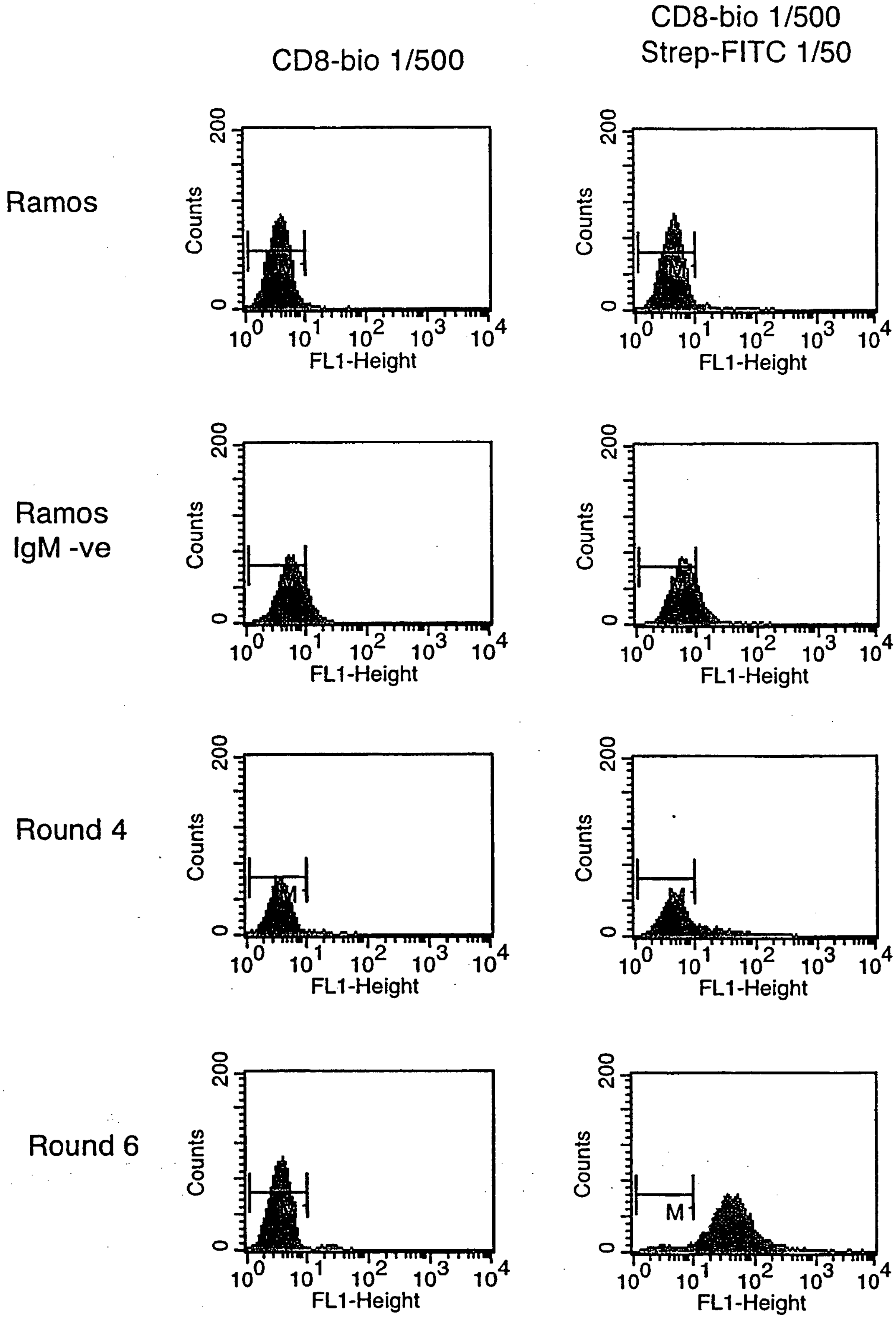

CD8-bio 1/500
CD8-bio 1/500
Strep-FITC 1/50
Ramos
Ramos
IgM -ve
Round 4
Round 6
Counts
FL1-Height
M

FIG. 15

FIG. 16

VH

```
1/1                                     31/11
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W   G   A   G   L   L   K   P   S   E   T   L   S   L   T   C   G   V   Y   G

61/21           CDR1                    91/31
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G   S   F   S   G   Y   Y   W   S   W   I   R   Q   P   P   G   K   G   L   E

121/41      CDR2                        151/51
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W   I   G   E   I   N   H   S   G   S   T   N   Y   N   P   S   L   K   S   R

181/61                                  211/71
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V   T   I   S   V   D   T   S   K   K   H   L   S   L   K   L   S   S   V   N

241/81                                  271/91  DJ
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A   A   D   T   A   V   Y   Y   C   A   R   V   I   T   R   A   S   P   G   T

301/101                                 331/111
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D   G   R   Y   G   M   D   V   W   G   Q   G   T   T
        AGC
        S
```

VL

```
1/1                                         31/11                         CDR1
CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC
P   A   S   V   S   G   S   P   G   Q   S   I   T   I   S   C   T   G   T   S

61/21                                       91/31
AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC
S   D   V   G   G   Y   N   Y   V   S   W   Y   Q   Q   N   P   G   K   A   P
        TTT TGT
        F   C
121/41              CDR2                151/51
AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC
K   L   M   I   Y   D   V   S   N   R   P   S   G   I   S   N   R   F   G   S
                    GCT
                    A

181/61                                      211/71
TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT
S   K   S   G   N   T   A   S   L   T   I   S   G   L   Q   A   D   D   E   A

241/81          CDR3                        271/91
GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC
D   Y   Y   C   T   S   Y   T   N   D   S   N   S   Q   V   F   G   G   G   T
```

FIG. 16CONT'D

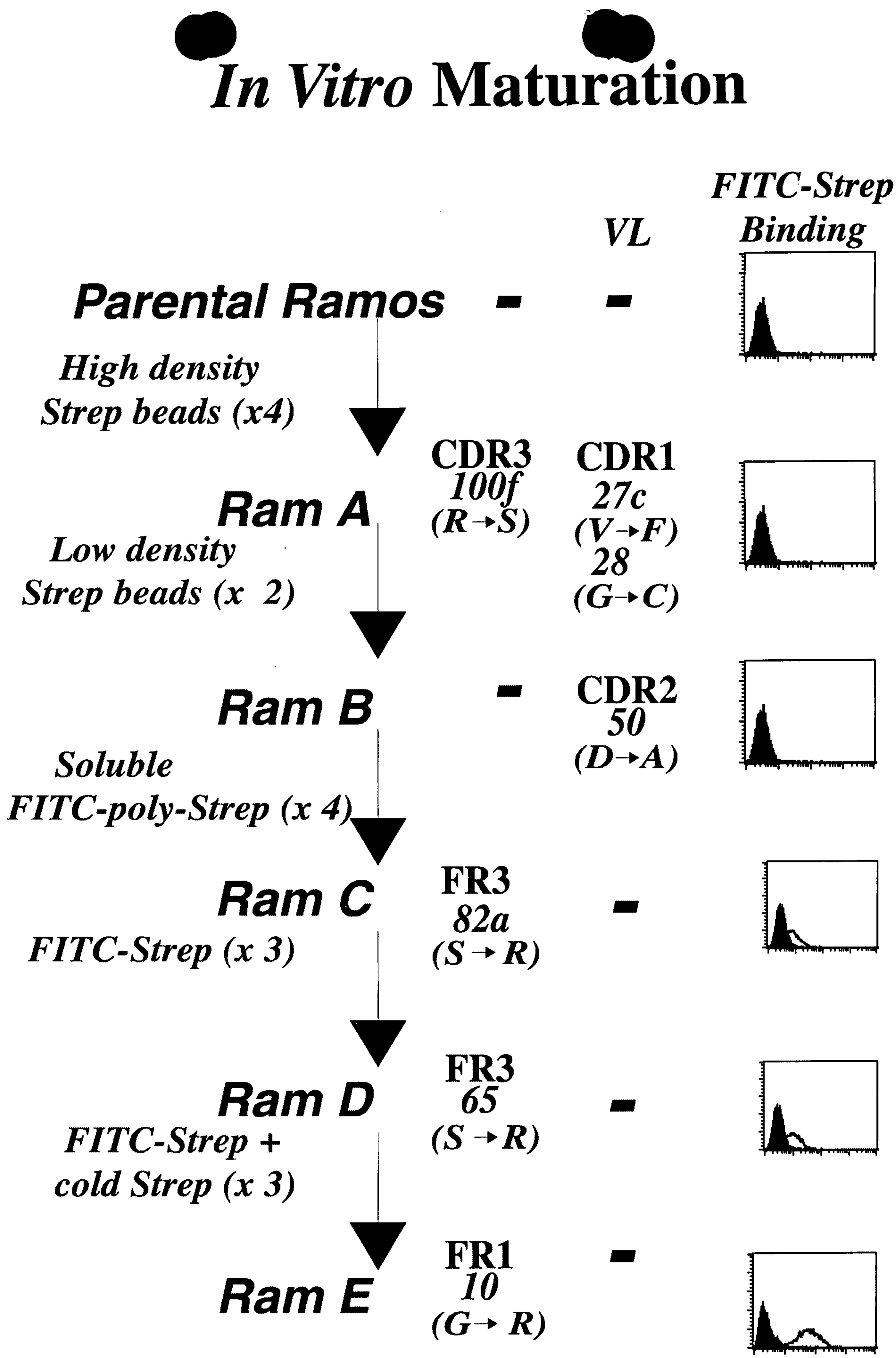

In Vitro Maturation
VL
FITC-Strep Binding
Parental Ramos - -
High density Strep beads (x4)
Ram A
CDR3 100f (R→S)
CDR1 27c (V→F) 28 (G→C)
Low density Strep beads (x 2)
Ram B -
CDR2 50 (D→A)
Soluble FITC-poly-Strep (x 4)
Ram C
FR3 82a (S→R) -
FITC-Strep (x 3)
Ram D
FR3 65 (S→R) -
FITC-Strep + cold Strep (x 3)
Ram E
FR1 10 (G→R) -

FIGURE 17

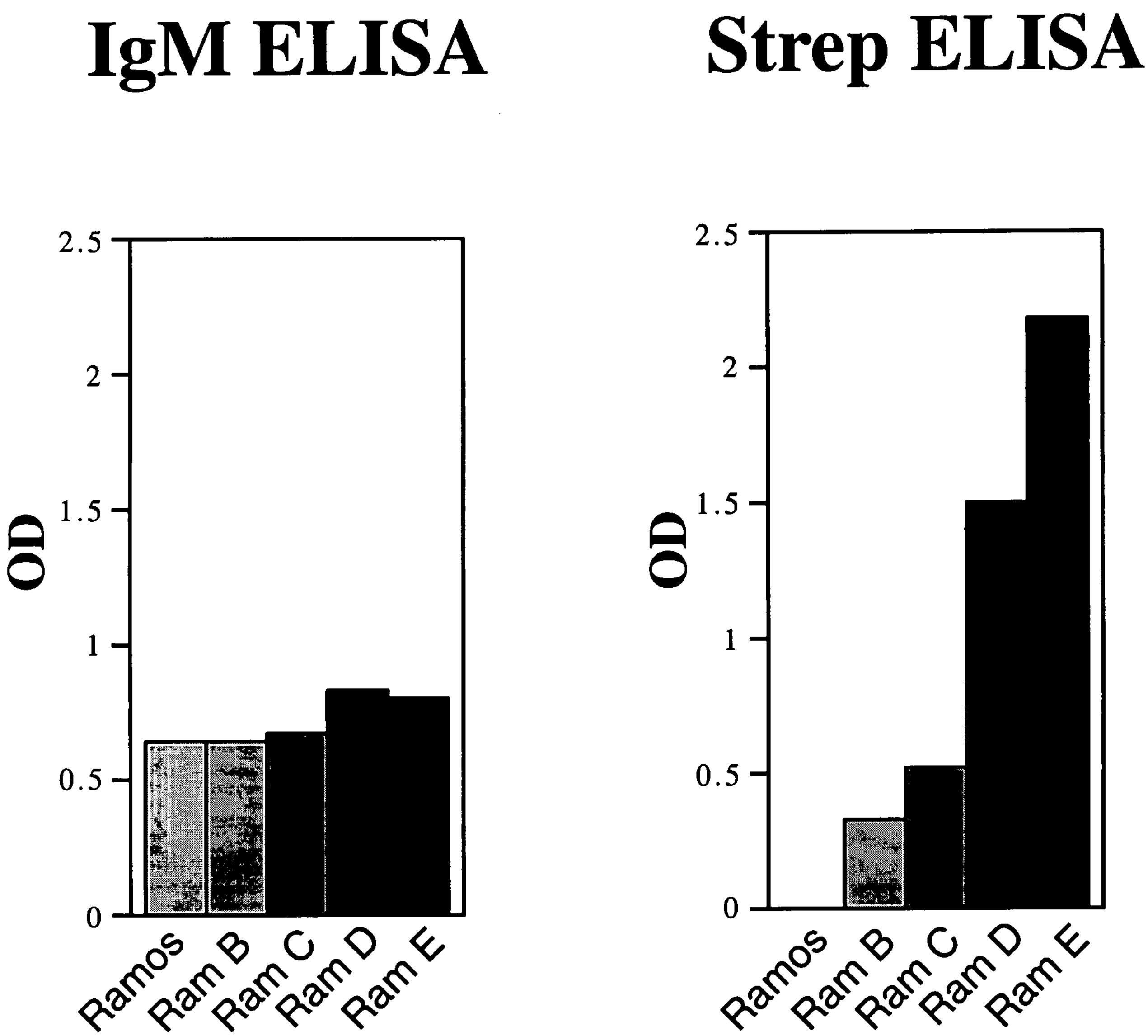
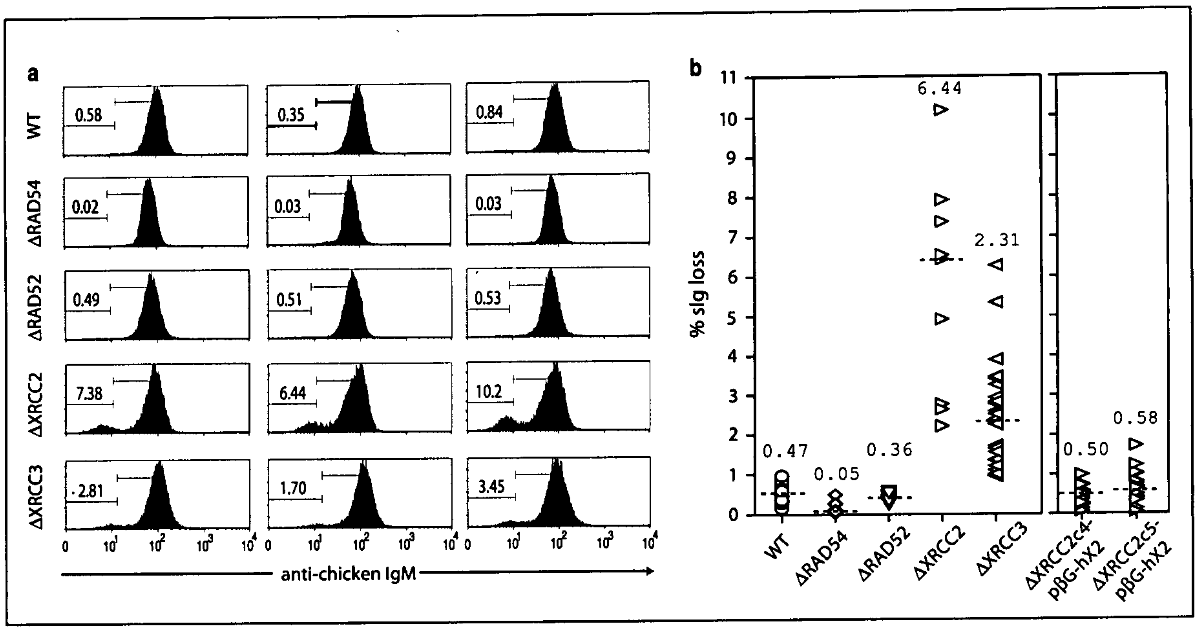
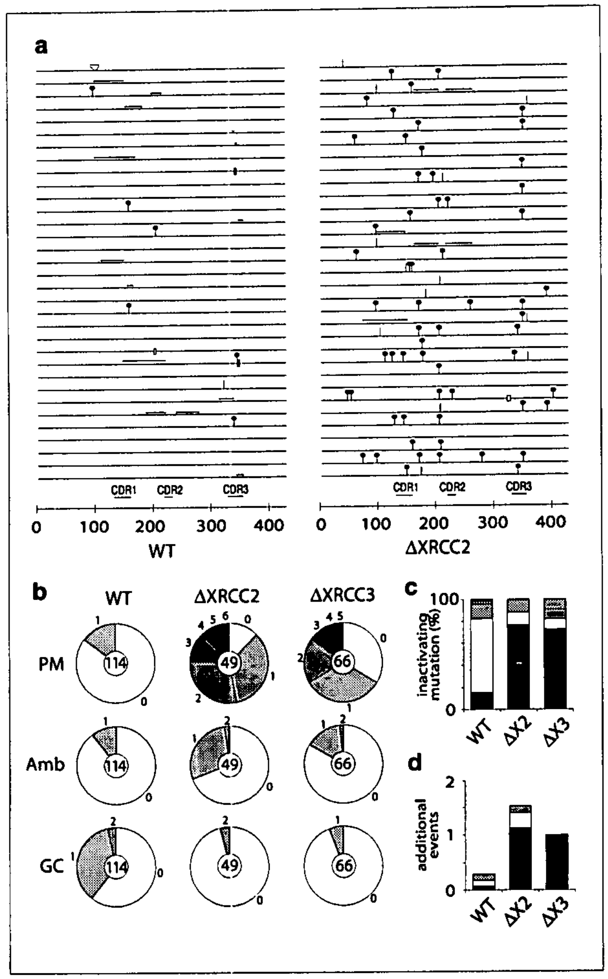

IgM ELISA
Strep ELISA
OD
2.5
2
1.5
1
0.5
0
Ramos
Ram B
Ram C
Ram D
Ram E

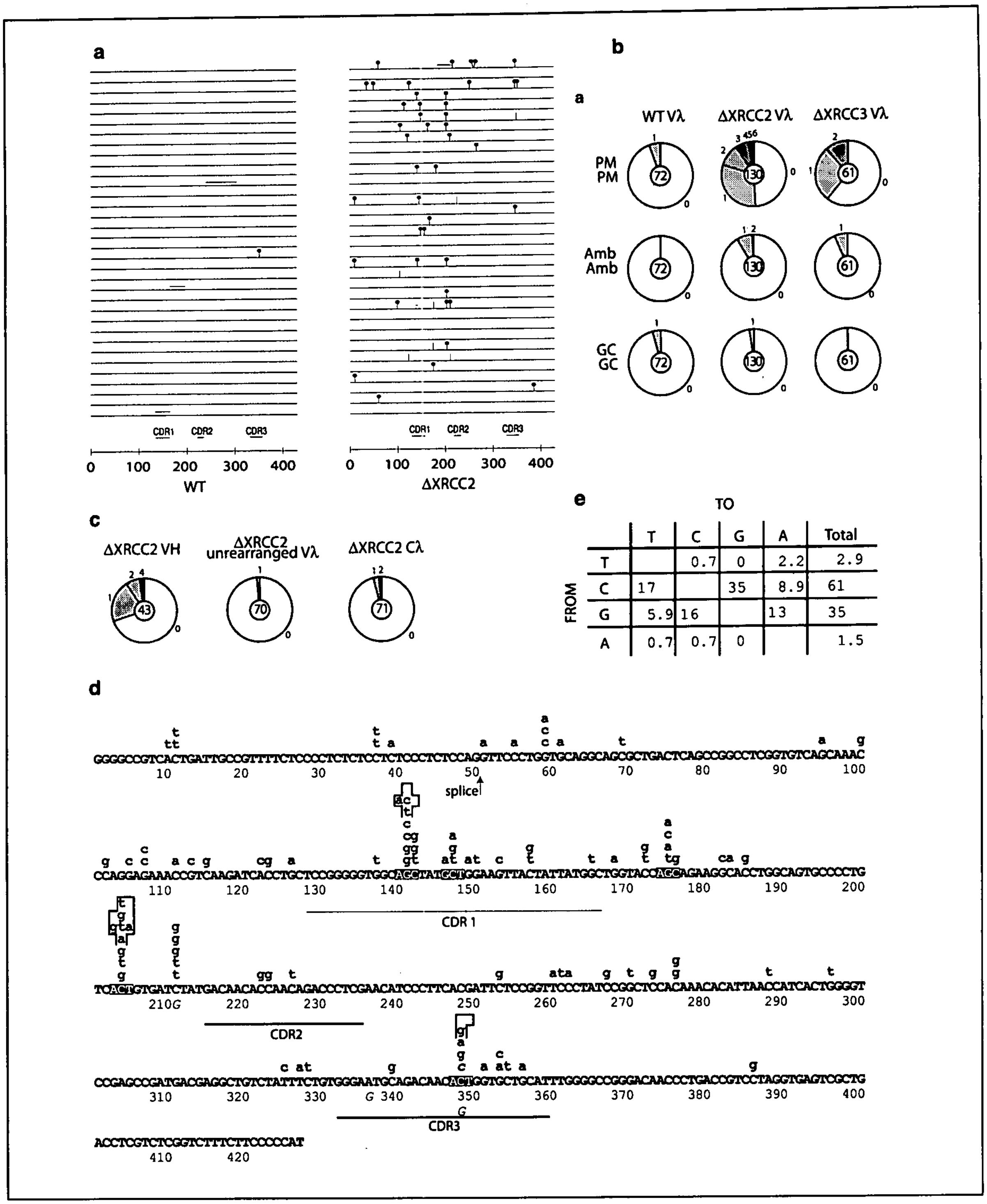
FIGURE 18



FIGURES 19A-B

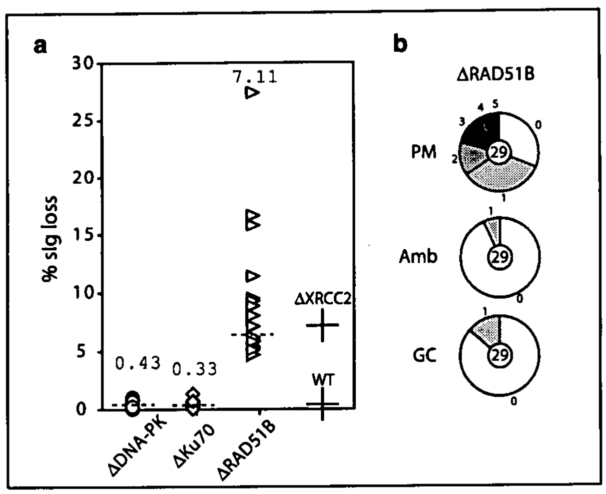


FIGURES 20A-C

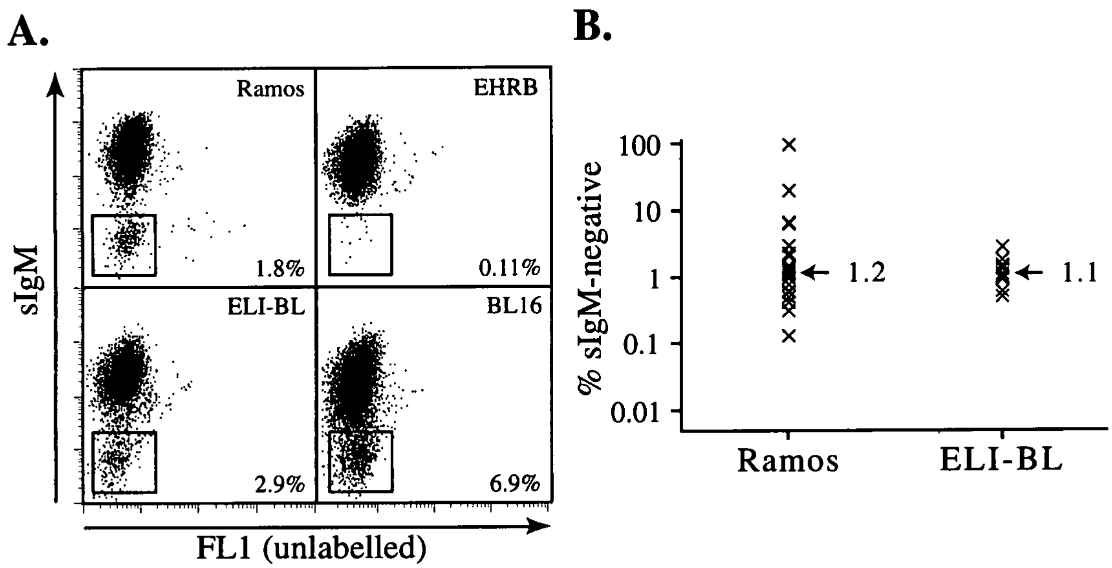


	T	C	G	A	Total
T		0.7	0	2.2	2.9
C	17		35	8.9	61
G	5.9	16		13	35
A	0.7	0.7	0		1.5

FIGURES 21A-D



FIGURES 22A-B



C.

																			t		
GTG	**CAG**	**CTG**	**GTG**	**GAG**	**TCT**	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	GGG	TCC	CTG	AGA	CTC	TCA	TGT	GCA
V	Q	L	V	E	S	G	G	G	V	V	Q	P	G	G	S	L	R	L	S	C	A
1									10										20		

				t			a ag	g													
GCC	TCT	GGA	TTC	ACC	GTC	AGT	AGC	AAC	TAC	ATG	ACC	TGG	GTC	CGC	CAG	GCT	CCA	GGG	AAG	GGG	CTG
A	S	G	F	T	V	S	S	N	Y	M	T	W	V	R	Q	A	P	G	K	G	L
							30										40				

	t						a a	c	ac	t	c	cg	t				t	cc			
GAG	TGG	GTG	TCA	CTT	ATT	TAT	AGC	GGT	GGT	AGC	ACA	ACA	TAT	TAC	GCA	GAG	TCC	GTG	AAG	GGC	CGA
E	W	V	S	L	I	Y	S	G	G	S	T	T	Y	Y	A	E	S	V	K	G	R
					50										60						

	t		c							t a						t	g	a			
TTC	ACC	ATC	TCC	AGA	GAC	AAT	TCC	AAA	AAC	ACG	ATG	TAT	CTT	CAA	ATG	AAC	AGC	CTG	AGA	GTA	GAG
F	T	I	S	R	D	N	S	K	N	T	L	Q	M	N	S	L	R	V	E	D	T
			70										80								

	t		a							a	t	a						g			
GAC	ACG	GCT	GTG	TAT	TAC	TGT	GCG	GGA	GAC	CTG	AAC	AGC	ACC	TCG	GTA	GGG	ACT	AAT	AAT	TTC	TAC
M	N	S	V	R	V	E	D	T	A	V	N	S	T	S	V	G	T	N	N	F	Y
	90										100										110

ATG	GAC	GTC	TGG	GGC	AAA	GGG	ACC	ACG	**GTC**	**ACC**	**GTC**	**TCC**	**TCA**
M	D	V	W	G	K	G	T	T	V	T	V	S	S
									120				

FIGURES 23A-C